DNA purification by centrifugation (Promega)

- First alternative:
  - Dissolving the Gel Slice
  - Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5 ml microcentrifuge tube.
  - Add 10 µl Membrane Binding Solution per 10 mg of gel slice. Vortex and incubate at 50-65 °C until gel slice is completely dissolved.
- Second alternative:
  - Processing PCR Amplifications
  - Add an equal volume of Membrane Binding Solution to the PCR amplification.
- Binding of DNA
  - Insert SV Minicolumn into Collection Tube.
  - Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
  - Centrifuge at 16,000 x g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
- Washing
  - Add 700 µl Membrane Wash Solution (ethanol added). Centrifuge at 16,000 x g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
  - Repeat Step before with 500 µl Membrane Wash Solution. Centrifuge at 16,000 x g for 5 minutes.
  - Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.
- Elution
- Carefully transfer Minicolumn to a clean 1.5 ml microcentrifuge tube.
- Add 15 µl of Nuclease-Free Water to the Minicolumn. Incubate at 60 °C for 5 minutes. Centrifuge at 16,000 x g for 1 minute. Repeat this step.
- Discard Minicolumn and store DNA at 4 °C or -20 °C.

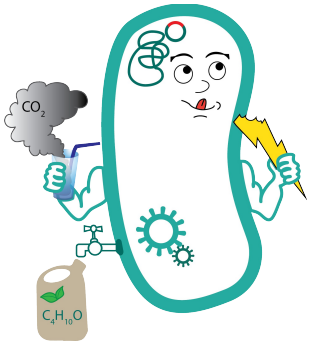
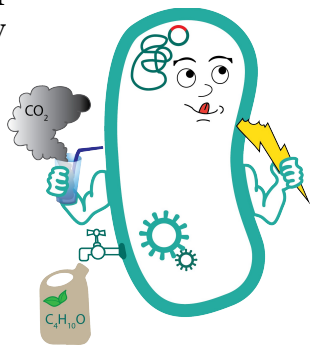


foto: sebastian kaulitzki

DNA purification by centrifugation (QIAGEN)

- **QIAquick Gel Extraction Kit by QIAGEN**
- Note: All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a conventional table-top microcentrifuge.
- Excise the DNA fragment from the agarose gel with a clean, sharp scapel.
- Wigh the gel slice in a colorless tube. Add 3 volumes Buffer QG to 1 volume gel (100 mg gel ~ 100 µl). The maximum amount of gel per spin column is 400 mg. For > 2% agarose gels, add 6 volumes Buffer QG.
- Incubate at 50 °C for 10 min (or until the gel slice has completely dissolved). Vortex thee tube every 2-3 min to help dissolve the gel. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose). If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The mixture turns yellow.
- Add 1 volume isopropanol to the sample and mix.
- Place a QIAquick spin colun in a provided 2 ml collection tube. To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of >800 µl, load and spin again.
- If the DNA will subsequently be used for sequencing, in vitro transcription, or microinjection, add 500 µl Buffer QG to the QIAquick column and centrifuge for 1 min. Discard flow-through and place the QIAqick column back into the same tube.
- To wash, add 750 µl Buffer PE o QIAquick column and centrifuge for 1 mi. Discard flow-through and place the QIAquick column back into the same tube. **Note:** If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), let the column stand 2-5 min after addition of Buffer PE. Centrifuge the QIAquick clumn in the provided 2 ml collection tube 1 min to remove residual wash buffer.
- Place QIAquick column into a clean 1.5 ml microcentrifuge tube.
- To elute DNA, add 50 µl Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAquick membrane and centrifuge the column for 1 min. For increased DNA concentration, add 30 µl Buffer EB to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min. After the addition of Buffer EB to the QIAqick membrane, increasing the incubation time up to 4 min can increase the yield of purified DNA.
- If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.



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